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Como Hacer un Asado

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Como Hacer un Asado

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For Mama and Papa

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I would like to thank...

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List of Abbreviations

mEC	**m**edial **E**ntorhinal **C**ortex
FOV	**F**ield **O**f **V**iew
DLC	**D**eep **L**ab **C**ut
BPFA	**B**eta **P**rocess **F**actor **A**nalysis
CNN	**C**onvolutional **N**eural **N**etwork
MSE	**M**ean **S**quare **E**rror
PSNR	**P**eak **S**ignal-to-**N**oise **R**atio
SSIM	**S**tructural **SIM**ilarity

List of Symbols

Global notations

I^{SR}	super-resolved light field image	—
I^{LR}	low-resolution light field image	—
I^{HR}	high-resolution light field image	—
E^{SR}	super-resolved epipolar plane image	—
E^{HR}	high-resolution epipolar plane image	—

Chapter 1

θ, ϕ	incoming direction expressed in term of spherical coordinates	rad
τ	time	s (second)
x,y	spatial coordinates with two-plane parameterization	1 (uint)
s,t	angular coordinates with two-plane parameterization	1 (uint)
P	radiance distribution	$\mathrm{W/srm^2Hz}$
Ω	image plane	—
Θ	parameters of the multi-layer framework	—
γ_s, γ_a	scaling factors of spatial / angular coordinates	1 (uint)
$\mathcal{L}$	loss function	—

Chapter 2

F_0	shallow features extracted by a single HConv layer	—
$F_{\mathrm{G_d}}$	feature maps extracted by the d^{th} HRB of the GRLNet	—
$H^{\mathrm{n}}_{\mathrm{HRB}}$	the operation of the n^{th} HRB of the SReNet	—
H_{AGBN}	the operation of the proposed aperture group batch normalization algorithm	—
H_{up}	upsampling operation on the low-resolution features	—
ℓ_A	angular loss	—
ℓ_S	spatial perceptual loss	—
ℓ_{SA}	the weighted combination of ℓ_A and ℓ_S	—

f	the summation of all the feature maps after every activation function of VGG network	—
g	learned mapping between the low-resolution and high-resolution light field images	—

Chapter 3

x,y	spatial coordinates with two-plane parameterization	1 (uint)
s,t	angular coordinates with two-plane parameterization	1 (uint)
γ_s, γ_a	scaling factors of spatial / angular coordinates	1 (uint)
ℓ_G	generator adversarial loss	—
ϕ	denotes the mapping of VGG network	—
δ	nearest neighbor downsampling operator	—
κ	a Gaussian blurring kernel with a window size of 7×7 and standard deviation of 1.2 pixels	—
η	additive noise with zero mean and unit standard deviation	—

Chapter 1

Introduction

1.1 Spatial navigation

The interaction between organisms and the environment is the core of life and evolution. This interaction happens at different levels with different objectives and outcomes. From the behavioural point of view, organisms have evolved to be able to perceive different aspects of the environment, interpret them and act in consequence. The more we move forward in evolution the more sophisticated and complex resources and behaviours we observe, being the nervous system, perhaps, the mayor and most interesting exponent of this.

One of the most fundamental aspects of such interaction consists on being able to move and navigate through the environment. A functional navigation system has to achieve a series of very difficult tasks that include the integration of information from different sensory modalities and coordination with the motor system, together with higher order cognitive processes like proprioception and goal directed activity in a flexible and dynamic way.

Spatial navigation has been extensively studied in the last 50 years, in particular, in the mammalian brain. The seminal work of John O'Keefe during the 1970s on rats lead to the hypothesis of a *cognitive map* [REF to the book] as the way the brain solves the challenge of spatial navigation and, importantly, to experimental corroborations of the possible neuronal implementation of it. Unexpectedly such implementation involved the activity of specialized cells in a restricted area of the brain: **The Hippocampus**.

According to the *cognitive map* theory, cells in the Hippocampus would receive inputs conveying information about sensory cues related to environmental stimuli, calculate the animal's position in space and consequently predict subsequent positions and trajectories depending on goal, inferred distances and directions. The ability of the internal navigation system to calculate trajectories and predict future positions represents the essence of learning in the cognitive map and has several implications regarding the internal structure, what kind of computations and types of cells should be find in the Hippocampus.

In the next sections we will summarize the anatomy and function of the Hippocampus and the different types of information encoding cells found to be present in the hippocampal navigation system.

1.1.1 The Hippocampus

Although hippocampal anatomy and connectivity has been extensively studied for decades, it's understanding and it's relationship with function is far from being completely elucidated [reference to something about discrepancies or open questions]. Here we will briefly described the canonical hippocampal circuit, it's constituents, structure and connectivity, paying special attention to the flow of information in the circuit.

The mammal Hippocampus is a seahorse-shaped (hence the name) brain structure located underneath the temporal lobe of the neocortex. All mammals have a structure that could be identify as an Hippocampus, moreover, it is possible to identify a homologue of the mammalian hippocampus in all vertebrates [reference to the okeefe book, Ariens Kappers, Huber, and Crosby 1936, pp. 1248-1255, Heier 1948, Crosby, Dejong, and Schneider 1966]. It's interesting to note though, that besides the difference in structure, the Hippocampus homologues can play an entire different functional role. The grid like structure of the Hippocampus could be thought as a general mapping structure that accomplish different functions depeneding on the species. In the mouse and rat, which is the case that concerns this work, is thought to be used as a spatial mapping structure, as discussed before.

In this animal the Hippocampus occupies a large portion of the forebrain and represents the paradigm of the simple cortex, consisting primarily of one basic cell type, the pyramidal or granule cells, and its asociated interneurons, the basket cells. In fact, a horizontal section through the posterior arch of the hippocampus shows the transition form the six layered complex structure of the entorhinal neocortex to the three layered hippocampal formation through the *subiculum*.

The hippocampal structure can be divided in two U-shaped interlocking sectors, the *hippocampus proper* and the *dentate gyrus*. The hippocampus proper can, in turn, be divided in 4 subfields CA 1-4 [Lorente de No 1934]. CA stands for *cornu ammonis*, another shape-like reference. Following the structured layer of principal neurons CA 1 appears first as the main output region of the hippocampus, followed by CA 2-3 in the regio inferior and finally CA 4 represents the scattered cells inside the hilus of the dentate gyrus. With the exception of CA 4, all regions of the hippocampus have a common simple structure: a compact and dense layer of cell bodies who's dendrites stretch in the same direction and receive most of their inputs from perpendicular running axons that make synapsis with many neurons at constrain regions of the dendrites. Such simple and preserved structure of the hippocampus represents one of the key aspects of it's function. The different subregions differ in the types of cells they have, CA 3 having giant pyramids, CA 1 smaller pyramids and granule cells in the dentate gyrus.

Internally the dentate gyrus has threer layers: the *granule* layer that contains the cell bodies of the mentioned granule cells, the *molecular* layer consisting of the apical dendrites of the granule cells and their afferents and finally the *polymorph* layer in the concave hilus of the dentate gyrus formed by the axons of the granule cells forming the mossy fiber bundle that merges with CA 4. Present in this last layer there're also some scattered basket cells interneurons. The hippocampus proper, although it's basically a three layered structure, it can be further divided for better describing the pyramidal cells and their afferents. First there's the *alveus* layer formed by the axons of the pyramidal cells that project to the subiculum, then we find the *stratum oriens* containing the basal dendrites, some basket cells and afferents from the septum. Third, the *stratum pyramidale* with the cell bodies and finally the *stratum radiatum* and the *stratum moleculare* with different parts of the apical dendrites. It's interesting to note that the main feature conveying the lamination of the hippocampal structure is the nature of their afferents, briefly described next.

The connectivity in the hippocampus is highly complex and the afferents arise from many different regions of the brain, here we will describe only the canonical circuit that can be described starting with the **extrinsic afferents**. The main source of input to the hippocampus is the entorhinal cortex that projects from its lateral and medial regions, passing by the upper layers of the subiculum, to either the hippocampus proper through the perforant path or to the dentate gyrus through the hippocampal fisure [REF Nafstad 1967, Hjorth-Simonsen and Jeune 1972, Van Hoesen, Pandya, and Butters 1972, Hjorth-Simonsen 1973, Van Hoesen and Pandya 1975b].

Once in the hippocampus the major **interconnections between sectors** are primarily unidirectional, starting from the dentate gyrus, through CA3 and ending in CA1 [REF Lorente de No 1934, Raisman, Cowan and Powell 1965, Hjorth-Simonsen 1973, Andersen, Blackstad, and Lømo 1966, Fujita and Sakata 1962, Gloor, Vera, and Sperti 1963]. Cells in the dentate gyrus have axons that gather together in the hilus forming the mossy fibers. The mossy fibers split in two bundles that project to the hippocampus proper. One bellow the pyramidal neurons in the stratum oriens, that stops abruptly in CA3. The second bundle runs above the pyramidal cells of CA3 through the stratum lucidum and continues until the border of CA1. CA3 and CA4 neurons make powerfull excitatory connections to the stratum radiatum of CA1 called *shaffer collaterals* [REF Lorente de No 1934, Hjorth-Simonsen 1973, Andersen, Blackstad and Lømo 1966]. Collaterals from CA3 and CA4, potentially the same that form the *shaffer collaterals*, bend and project back to the proximal dendrites of the granule cells in the dentate gyrus [REF Zimmer 1971]. It is believed that CA1 does not project back to CA3 [REF Raisman, Cowan, and Powell 1966, Hjorth-Simonsen 1973] but it is unclear if it projects to the dentate gyrus [REF Hjorth-Simonsen 1973]. Interestingly CA1 and the dentate gyrus receive inputs from CA3 of both hippocampi, including the contralateral one. Then the information flows out of the hippocampus by CA1 cells axons that project to the septum and to the subiculum which in turn projects to back to the entorhinal cortex, closing the loop in the information flow.

Finaly, there's the **intrinsic afferents from the same sector,** that is, within each region of the hippocampus there's local connectivity in two flavours, excitatory monosynaptic connections between close by pyramidal neurons [REF Lebovitz, Dichter, and Spencer (1971)] and inhibitory polysynaptic connections due to the instrinsic pyramidal - interneuron - pyramidal loops, where the interneurons are the basket cells mentioned before [REF Kandel,Spencer, and Brinley 1961, Spencer and Kandel 1961c, Andersen, Eccles, and Løyning (1964a,b)].

To complete this brief description of the calssical hippocampal circuit we have to mention that the entorhinal cortex in turn receives a plethora of inputs from different parts of the brain, among which there are the prefrontal and cingulate cortices [REF Adey 1951, Adey and Meyer 1952, White 1959, Cragg 1965, Raisman et al. 1965, McLardy 1971, Leichnetz and Astruc 1975], the temporal cortex [REF Cragg 1965], parietal areas [Pandya and Kuypers 1969, Pandya and Vignolo 1969, Petras, 1971], pyriform cortex [REF Powell, Cowan, and Raisman 1965], the olfatory [Cragg 1960, 1961, Heimer 1968, White 1965, Price and Powell 1971, Kerr and Dennis 1972] and visual systems [REF Casey, Cuenod, and MacLean 1965, Cuenod, Casey and MacLean 1965] and the amygdala [(Krettek and Price 1974].

This is by no means a full description of the hippocampal connectivity and its afferents, but only a succinct description of the canonical pathway through which information flows in the circuit. In this description information flows from several regions of the neocortex and other brain region to the entorhinal cortex and subiculum, from here to the dentate gyrus, then to CA3-4, finalizing in CA1 that projects back to the subiculum and entorhinal cortex (and to the septum) closing the loop. Interestingly, the projections in this path are topographically precise, in the sense that, for example, a small number of cells in the dentate gyrus projects to a small number of cells in CA3.

How does such a precise and well define anatomy and connectivity structure solve the problem of spatial navigation? When O'Keefe first elaborated the *cognitive map* theory he hypothesized that each of the three regions of the hippocampus accounted for a stage in the mapping system [REF O'keefe book]. The first stage, occurring in the dentate gyrus, would consist in organizing the environmental inputs from the entorhinal cortex and subiculum into a schema required by the mapping system. This complex integrations would then be transmitted to CA3-4 where the second stage of the map would take place, by representing locations in an environment and the relationship between locations. Finally in CA1 the continuation of the map would be represented together with a mismatch system that would account for novelty or change in location information.

This very simple schematic turned out to be highly accurate in some senses and the last 4 decades of experiments have come up with empirical evidence of implementations of such system. The scheme has been improved and completed over the years, the current understanding in the field includes the subiculum and entorhinal cortex as part of the greater hippocampal formation. And in each of these regions it has been

found a set of highly specialized neurons that together form the spatial map. Many of them have been predicted in the '70 by the *cognitive map* theory. According to it cells that encode position, distance and speed would be necessary. In the next section we'll summarize the main types of cells of the circuit and how they fit in the overall scheme.

1.1.2 Spatial information encoding cells

The first type of cells that conform the cognitive map were found by O'Keefe and Dostrovsky in 1971, when electrophisiological recordings in the hippocampus led to the discovery of cells that would fire predominantly in a specific location of a familiar environment. This cells were called *Place cells*. It's impossible to summarize here all what is currently known about place cells, so we will focus only in the main characteristics of this and the rest of the cell types of the cognitive map.

Place cells are mainly found in the hippocampus proper and their firing rate is modulated purely by spatial location, that is, they fire maximally when the animal's head is in a specific region of the environment. This region is called the *place field* of the cell. Here we use the term environment in a generic way, but place cells have been mainly studied in constrained laboratory environments. Interestingly, place cells have different characteristics depending on the nature of the space the animal explores. Place cells fire at their place field location, regardless of directions of motion or speed when the animal is in a two-dimensional space, like the square/rectangular or circular boxes used in the early O'keefe experiments, but when exposed to a linear track, or one-dimensional environment, place cells would have a preferred direction of firing, and would fire much less or not at all or in a different location when the animal runs in the oposite direction [McNaughton et al., 1983; O'Keefe and Recce, 1993]. Place cells have also been found in three-dimensional environments, having three-dimensional place fields. The latter type of place cell has been observed in bats flying through a familiar environment [Yartsev and Ulanovsky, 2013], and more recently in rodents exploring three-dimensional environments [Grieves...Jeffery 2020].

The way place cells represent position is not limited to the firing rate of the cells but also to the temporal aspect of their firing. Place cell firing is locked to the phase of the sinusoidal local field potential (LFP), called theta rhythm, and hence to the population activity in the hippocampus. The theta rhythm works as a sort of clock against which the network can measure time and temporally locate cell spikes allowing place cells to identify locations in an environment with much finer precision than if only rate codes were used. This is called *Temporal coding* [O'Keefe and Recce (1993), Huxter et al., 2003, György Buzsáki, Andreas Draguhn 2004, Buzsáki 2002].

Nearby place cells do not necessarily have nearby place fields, furthermore, a group of close by place cells would typically have place fields that span the whole space, suggesting that place cells represent a complete and highly redundant representation of the surface [O'Keefe, 1976; Wilson and McNaughton, 1994]. Once formed, these representations are stable across days [Hill, 1978; Muller et al., 1987] or even

weeks [Thompson and Best, 1990]. Although, more recently, it has been suggested that not all place fields are stable [Mankin et al., 2015; Ziv et al., 2013].

There's a large portion of literature related to what are the necessary inputs to the hippocampus for a place cell to fire. This is still an unsolved question, althought there are some clear hints. It is clear that visual information is important, as distal cues or landmarks surrounding the environment can influence place field formation [Muller and Kubie, 1987; O'Keefe and Conway, 1978; Yoganarasimha and Knierim, 2005] but it is not necessary. Place cells would fire in the same location the dark in a familiar environment [Save et al., 2000; Zhang et al., 2014; Markus et al., 1994; Quirk et al., 1990], provided that other sensory cues are avialable such as olfaction or tactility. All this different modalities are integrated outside of the hippocampus [Jeffery, 2007], which shows that place fields are higher order representations that integrate more primitive spatial constructs such as direction, self motion and boundaries, which again talks about the inputs to the hippocampus. If the sensory cues show that the environment has changed or is completly new, a new and unique representation would be formed by the place cells [Anderson and Jeffery, 2003; O'Keefe and Conway, 1978] in a process called remapping [Muller and Kubie, 1987]. Importantly, for a map to be formed in rats and mice the animal has to explore the space directly for place cells to form a spatial representation [Rowland et al., 2011], unlike other mammals like primates that can form inferred allocentric representations of remote space if observed [Rolls, 1999; Rolls et al., 1997; Rolls and O'Mara, 1995]. So far we've used a rather vague definition of place cell. Traditionally, to define a place cell and its place field several criteria related to the firing rate, consistency of firing or reliability were used. Here and throughout this work we will define a place cell as a cell in the hippocampus proper that carries *significant amount of information* about the animals position in it's firing activity (see methods). Later on we will define what this means in this context and how we establish significance.

The second key cell type in the spatial representation are the **head direction cells**. Head direction cells are cells in the presubiculum whose firing is modulated, as the name implies, by the facing direction of the head. They were first found by Rank [Rank 1985, 1984] and described in detailed a few years later [Taube et al., 1990a, 1990b, 1987]. Here *head direction* refers to the orientation of the head in the horizontal plane. Head direction cells are very similar in their characteristics to place cells: they have a prefer direction of firing that is independent of other behavioural factors; each head direction cell has a different preferred direction; all together, preferred directions are equally distributed in the circle, in the sense that there's no overall preferred direction of the network [Taube et al., 1990b]. Like with place cells, angular orientation of environmental cues are an important modulator of head direction cells activity [Goodridge and Taube, 1995; Taube, 1995a; Taube et al., 1990b; Zugaro et al., 2000Knierim et al., 1995] but are by no means necessary [Mizumori and Williams, 1993; Yoder et al., 2011a,b]. An interesting characteristic of this cells is that the angular relationship between preferred directions of different cells is preserved [Skaggs et al., 1995; Yoganarasimha and Knierim, 2005]. Hence when remapping an environment or if the animal is disoriented

and one cell changes its orientation, the rest of the cells change theirs coherently.

With place cells and head direction cells, the cognitive map is able to build positions and to measure angles. The next requirement for the map to work is a way of measuring distances, to establish the metric of the map. In 2005 a cell type that could achieve this task was found in the Moser's lab: the **grid cells**. Grid cells are cells that fire in multiple discrete and regularly spaced locations which form a triangular or, equivalently, an hexagonal lattice. This cells are found in the medial entorhinal cortex (mEC) and postrhinal cortex [Fyhn et al., 2004; Hafting et al., 2005, Fyhn et al., 2008] and in the pre- and para-subiculum [Boccara et al., 2010].

Grid cells have some similar characteristics to place cells or head direction cells. Their pattern of firing arises in familiar environments and partially relies on distal visual cues, if the environmental cues rotate, grid patterns do so too consistently [Hafting et al., 2005], and deformation of the environments implies deformation of he patterns [Barry et al., 2007; Stensola et al., 2012]. Like head direction cells, the angles and distances between grid patterns of different grid cells are preserved, and when the environment rotates or moves, the patterns adapt in a coherent fashion, mantaining a stable relationship [(Fyhn et al., 2007]. This suggests that grid cells work cooperatively, as an interconected matrix known as attractor network [McNaughton et al., 2006]. Moreover, the spacing between peaks of grid patterns varies as a function of location in the entorhinal cortex. The scales of the patterns increase in discrete jumps as one goes from dorsal to ventral in the entorhinal cortex [Vegard Heimly Brun, et al. 2008]. Each animal can have 3 or 4 different scales.

Finally, we have the **boundary cells**. With yet another highly descriptive name, boundary cells, or boundary vector cells, are cells in the subiculum that respond purely to environmental boundaries. Interestingly, the existence of boundary cells was first hypothesized after the observation that after elongating one side of a rectangular box, place fields would stretch accordingly [O'Keefe and Burgess, 1996]. This led a number of researchers to think that there could exist cells that would fire in relation to environmental boundaries, and that place cells firing could arise as a thresholded sum of a subpopulation of such cells [Barry et al., 2006; Burgess et al., 1997; Hartley et al., 2000]. Cells that fit such description, at least partially, were later found in several regions of the brain, like the subiculum [Barry et al., 2006], presubiculum and parasubiculum [Boccara et al., 2010], mEC [Bjerknes et al., 2014; Savelli et al., 2008; Solstad et al., 2008] and recently in the anterior claustrum [Jankowski and O'Mara, 2015] and rostral thalamus [Jankowski et al., 2015].

More formally we could define boundary vector cells as cells that fire when the animal encounters an environmental boundary in it's preferred direction. And it's firing is driven by the memory of the boundarie's position related to the animal, based not only on perceptual cues, but also on self motion information [Lever et al., 2009; Raudies et al., 2012; Raudies and Hasselmo, 2012]. This definition requires that we clarify two things: first what is a boundary? A boundary can be walls, low ridges or vertical drops

and the colour, texture or odour of these does not seem to influence the cell's firing [Lever et al., 2009]. Second what does it mean to *encounter* a boundary. Cells would fire at a specific disntace from the boundaries, and this distance is different for cells in different brain regions [Bjerknes et al., 2014; Solstad et al., 2008, Jankowski and O'Mara, 2015, Lever et al., 2009].

Place cells, head direction cells, grid cells and boundary vector cells lie at the core of the cognitive map and represent the most relevant more studied type of cells in the context of spatial navigation. However the further the cognitive map and the greater hippocampal formation is studied, the more *types* of cells are found. Cells with more abstract or complex firing patterns, cells that respond to clear real-world correlates, but also cells that respond to more abstract or conjunctive correlates. We will not describe them here, but in this list we should mention **object cells**, **goal cells**, **boundary-off cells**, **perimeter cells** and **band cells**, among others.

It's interesting to think how each of this cell types can arise, due to which inputs, and in which combinations. In other words, what is the relation between the firing patterns of all these cell types? Mathematically is easy to show that place cells can be formed by summing two grids of different spacing, or equivalently by summing two border cells, or that grid cells can be built by combining band cells. The function and structure of each of this firing patterns is not yet understood. It is however almost difficult to believe that the brain builds such an explicit and interpretable map, using specialized cells in trackable combinations. This is of course, just the tip of the iceberg and the more the extended hippocampal formation is studied, the more cell types and complicated firing patterns appear.

However, as intense and productive all the before-mentioned (and much more that has not been mentioned here) research has been in the last decades, we can't help but to notice that it concerns only a subgroup of the full brain network: the neurons. But neurons represent approximately half of the cells in the brain, depending on the species can be more or less, the rest are **Glia cells**. Although Glia cells don't have electrical activity like neurons have, they express rich calcium activity and interact with cells in active ways. In this work we will approach the question of if and what role play glia cells in spatial navigation in the mouse brain.

But first we will briefly describe the types of Glia cells that can be found in the brain, focusing specially on **Astrocytes**, the main actor of this work, their characteristics and the recent literature regarding its calcium signaling and its role in modulating neuronal activity.

1.2 Glia

Glial cells have been first observed as early as the mid 19^{th} century by Virchow [Virchow, 1856], and better described and brought to wider attention by Santiago Ramón y Cajal and Pío del Río Hortega a few decades later thanks to the development of

chloride-sublimate technique, a staining technique that targets specifically astrocytes. At the time, glial cells were thought to play a strictly structural role in the brain. If anything else, the terminology used to describe them would be sufficient to understand the hypothesized role: described as *Zwishchenmass*, german for *inbetween mass*, *Nervenkitt*, or *nerve glue* in english, and finally the current terminology *Glial cell* comes from the Greek word *glía* meaning *glue*. It wasn't until the second half of the 20^{th} century when electrophysiological characterization and physiological studies of glial cells permitted the understanding of the wide range of vital functions that glial cells have in the functioning of the central nervous system [Morrison and de Vellis, 1981, Bowman and Kimelberg, 1984; Kettenmann, Backus and Schachner, 1984, Cornell-Bell et al., 1990a, Araque et al., 1998; Bezzi et al., 1998]. Phylogenic studies show that all organisms with a central nervous system have glial cells, and, interestingly, the ratio of astrocytes-to-neurons is different depending on the animal species and on the brain region, with intriguing correlates with brain complexity and neuronal density [Herculano-Houzel, 2011, Herculano-Houzel, 2014]. Throughout evolution, glial cells have diverge into specialized subgroups with different characteristics and function. The total glial population can be divided into four major groups: **microglia**, **astrocytes**, **oligodendrocytes** and their progenitors **NG2-glia**.

Unlike the rest of the glial cells, **Microglia** originate from yolk-sac progenitors that only populate the brain during development [reviewed in Kim and de Vellis, 2005; Kettenmann et al., 2011]. They represent the main immuno-competent and phagocytic cells of the central nervous system [Filiano AJ, Gadani SP, Kipnis J August 2015], and cover the major part of adult brain in individual non-overlapping domains. Microglia sense the environment through the movement of their filopodia, which rapidly reacts to abnormalities or damage [Nimmerjahn et al., 2005; Cronk and Kipnis, 2013]. Besides the inmuno-role, microglia has recently been hypothesized to have an active role in the healthy brain. Opinions on this matter are , however, controversial. While some studies show that microglia could be involved in motor-dependent synapse formation [Parkhurst et al. (2013)] and in features as high order as learning or social behavior [Torres et al., 2016, Kierdorf and Prinz, J Clin Invest. 2017], others have shown that ablation of microglia barely produce any alterations or pathologies in healthy adult mice [Elmore et al., 2014, 2015; Bruttger et al., 2015]. This discrepancies might be due to the major methodological differences in each study [Sarah Jäkel,and Leda Dimou 2017].

Oligodendrocytes are a type of large macroglia cells first observed by Pío del Río Hortega in the first half of the 20^th century. Their function is somewhat more clear: they insulate axons with self-produce myelin to allow a fast saltatory conduction and give trophic support to axons [reviewed in Nave, 2010]. However oligodendrocytes have been found in sparsely myelinated brain regions, this presumably non-myelinating oligodendrocytes might have other functions that have been so far overlooked.

More interesting are the more recently discovered oligodendrocytes precursors,

the **NG2-glia** cells [ffrench-Constant and Raff, 1986]. Their first more evident function is that of forming and maintaining a homeostatic network, preserving the cell numbers stable by generating mature myelinating oligodendrocytes throughout lifetime [Dimou et al., 2008; Rivers et al., 2008; Psachoulia et al., 2009; Simon et al., 2011, Hughes et al., 2013] under physiological conditions. What's really interesting about the NG2-glia cells is their ability to form functional synapses with neurons. A fenomena first observed in the hippocampus [Bergles et al., 2000] but later described in other brain regions [Karadottir et al., 2005; reviewed in Sun and Dietrich, 2013]. Such synapses are uniderectional in the sense that can only receive neuronal sygnals but can't generate action potentials on their own and further propagate them [De Biase et al., 2010].

The last large group of glia cells in the brain are the **Astrocytes**. Astrocytes and the effect of alterations on their calcium activity represent the main focus of this thesis. For this reason we will spend the next few sections on describing their function, anatomy and their known relation with neuronal activity.

1.2.1 Astrocytes

Astrocytes are the most abundant type of glial cell and represent up to 40% of all the cells in the mammalian brain [Herculano-Houzel, 2014]. Despite being one of the first glial cells to be discovered around 150 years ago, their description and the understanding of their role in the brain function is far from complete. As with everything in biology (is getting annoying really), astrocytes do not represent single homogeneous cell type and can be subdivided into several types depending on their morphology, molecular profile or function.

From the morphological point of view astrocytes can be roughly divided into two types: **fibrous** and **protoplasmic**. The first one is a star-shaped cell with regular contours present mainly in the white matter of the brain and spinal cord and in the optic nerve and the retina fiber layer. Fibrous astrocytes are characterized by their elongated morphology, with long processes running parallel to the axon bundles that make contact with myelinated axons and with oligodendrocytes. They have fewer processes compared to protoplasmic astrocytes. Their processes spatially overlap in their domains and extend to perivascular, subpial and axonal endfeet [Lundgaard et al., 2014].

Protoplasmic astrocytes on the other hand have a "bushy" and irregular morphology, with a small round somata of $\sim 10\mu m$ in diameter. Present $5-10 \sim 50\mu m$ primary processes, that further branch into thousands of branchlets and leaflets that form dense arborisations that connect with synapses [Bushong et al., 2002], and large endfeet that in turn connect with the vasculature [Nagelhus and Ottersen, 2013; Verkhratsky, Nedergaard and Hertz, 2015]. Unlike fibrous astrocytes, protoplasmic astrocytes populate mainly the gray matter in the brain and have domains with well defined borders that do not overlap between each other [Bushong et al., 2002]. Even when the the area of influence of an astrocyte is limited to local domains and do not mix with other astrocytes,

it is highly connected and has a strong influence in neuronal activity. A single astrocyte arborisation can cover 20,000 to 80,000 μm^3, contacting 300 to 600 dendrites and potentially 100,000 individual synapses [Bushong et al., 2002, Halassa et al., 2007]. This dense connectivity allows astrocytes to control several processes like ion homeostasis or neurontransmitter recycling. Interestingly, astrocytic domain boundaries have been proposed to be determined by, or at least closely relate to, neuronal functional units [Perea, Sur and Araque, 2014]. In this sense astrocytes could play the role of controlling and modulating *functional islands* formed by the synapses confined within the area of influence of a single astrocyte [Halassa et al., 2007]. Further supports this hypothesis the fact that branching and connectivity of astrocyte, even from the same type, strongly depends on brain region. When comparing striatal and hippocampal astroglial populations it was noted that, despite having the same somatic volume, equivalent number of primary branches, and the same total cell volumes, hippocampal astrocyte territories are more constrained and display a tighter physical interaction with excitatory synapses [Chai et al., 2017] compare to striatal ones.

If astrocytes are so closely related to neuronal function and, as said before, have a big and dense areas of influence, what are astrocytes functions in the brain? This question represents still a very active area of research. Here we will enumerate some of the known functions that astrocytes fulfill but will later describe in more detail the role of astrocytes in modulating neuronal activity.

Control of cerebral blood flow Metabolic support In Neurotransmitter clearance Potassium buffering

Establishment of neuronal circuits: synapse formation, stabilisation and pruning Gliovascular coupling, metabolic and immune support Ion, water and neurotransmitter homeostasis

Function and anatomy. Refer to papers by *Bushong, Fellin-Halassa, Shigetomi*

1.2.2 Calcium signaling in astrocytes

Refer to paper by *Barzagani Attwell*. Most of literature comes from calcium buffers to measure calcium, maybe consider some modeling works.

1.2.3 Astrocityc modulation of neuronal activity

1.2.4 Astrocytes accumulate evidence

Refer to paper from *Misha Harens*

1.3 Astrocytes encode spatial information in Ca^{2+} activity

1.3.1 Astrocytes and information

Treating astrocytes Ca^{2+} activity with Information theory approaches

1.3.2 Decoding of position

Chapter 2

Rational and Aim

Maximum one page, just to state the logical flow in a few sentences and what we gonna do. We know that:

- Astrocytes encode spatial info in Ca2+ activity
- Ca2+ in astrocytes modulates neuronal activity

That brings us to the questions:

- What is the functional role of the Ca2+ signaling in astrocytes in spatial information encoding?
- Does modulation of Ca2+ in astrocytes alter spatial encoding in neurons? how?

To adress this questions we used 1p and 2p imaging + cell specific chemogenetic manipulation in the mouse hippocampus.

Chapter 3

Materials and Methods

3.1 Experimental procedures

How was the experiment performed? (briefly) animals, surgeries, 1p/2p imaging.

3.2 Data pre-processing

In this work we dealt with diverse and complex datasets, of different types and structures and therefore, that require different pre-processing pipelines. Here the details for each case.

3.2.1 Inscopix 1-photon imaging

The Inscopix software acquires imaging data from miniscopes in freely moving animals. The imaging data is then exported as *.isxd* files containing the images and the metadata. Such files can only be read and treated with Inscopix own proprietary software (**poner el link**), the following pre-processing was performed using such software.

All five imaging series corresponding to the same animal in the same day were first concatenated, cropped and downsampled in space and time. Temporal downsampling works by averaging n adjacent frames, where n is the temporal downsample factor. The moving average stride is equal to the temporal downsample factor, which results in non-overlapping groups of frames to be averaged. This is equal to binning the frame data in time (in bins defined by the temporal downsample factor) and the subsequent averaging of each bin. The resulting number of frames equals the original number of frames divided by the temporal downsample factor, rounded down. Spatial downsampling works similarly, except that the spatial bins are non-overlapping sub-images of the original frames. For all recordings we used a temporal and spatial downsample factor of 2 and 4 respectively. Both downsampling stages were used to be able to realistically mange data size and computation time.
To remove defective pixels a 3x3 median filter was applied to the movies, and early frames which were dark or dim were trimmed.

A spatial filter algorithm was then applied to each movie to remove low and high spatial frequency content. In practice the algorithm bandpass the images by convolving each frame with a gaussian kernel and subtracting a smoothed version of the frame from a less smoothed version of the frame. Parameters of the bandpass filter were set to **Low cut-off** $= 0.005\ pixel^{-1}$ and a **High cut-off** $= 0.5\ pixel^{-1}$.
Then each concatenated recording was motion corrected to compensate for unwanted motion of the brain relative to the skull. For each frame of the movie, motion correction estimates a translation that minimizes the difference between the transformed frame and the reference frame, using an image registration method described in *REF [Thevenaz1998]*.
Then the fluorescence in each pixel was normalize by the average fluorescence across frames to obtain the $\Delta F/F$, so that it represents a deviation or change from a baseline.

3.2.2 2-photon imaging

Data extracted using 2-photon microscopy produces t-series consisting of sequential *.tiff* images. All images corresponding to a t-series were first concatenated to produce an *.avi* video with no compression.
Motion corrected was then performed using the *NoRMCorre* algorithm *(REF Pnevmatikakis and Giovannucci, 2017)*, that corrects non-rigid motion artifacts by estimating motion vectors with subpixel resolution over a set of overlapping patches within the FOV. These estimates are used to infer a smooth motion field within the FOV for each frame. The inferred motion fields are then applied to the original data frames.
Motion correction was applied in two steps, first each t-series was motion corrected. Then, all t-series corresponding to the same day and same animal were concatenated and motion corrected again.

3.3 Animal tracking

3.3.1 Linear track

Animals performing the linear track task were head fixed and able to run in a wheel. The position of the animal is then considered as the position of the avatar in the virtual reality linear track. **PONER LA PARTE DE LOS METODOS DE SEBA**

3.3.2 2-D arena

In the random foraging and open field experiments in the 2-dimensional arena animals were free to move and explore a $45cmx45cm$ square box. The box was filmed using a **CAMARA MODEL** placed at **DISTANCE** meters from the floor.
Animal position was estimated from these videos using the software package **DeepLabCut** (DLC) **Link to the githubpage**.
DLC is a free software 000000000000000000000000000000000000

3.4 Video Segmentation

3.5 Trace extraction

3.6 Event detection

3.7 Place Cell detection

3.8 Statistical testing

3.9 Decoding of position from neural activity

3.10 Dimensionality reduction

Chapter 4

Results

4.1 Random Foraging

- Significant decrease in information content in place cells
- Differences in place fields properties (?)

4.2 Open Field

- Significant decrease in information content in place cells
- Differences in place fields properties (?)

4.3 Linear track

- No significant difference in information content
- Difference in place cell and place field properties, maybe as a function of reward and/or position in track

4.4 Population codes

- Decoding of position from neural activity
- Dimensionality reduction

Chapter 5

Discussion

Appendix A

About Appendix

The appendix is usually used to provide some supplementary materials for the publications. For example, some experimental results, network architecture, detailed experimental settings or proving of the theories. You can have more than one appendices to provide the materials for different uses.

Appendix B

Appendix Title Here

Write your Appendix content here.

Appendix C

Appendix Title Here

Write your Appendix content here.